APPENDIX B.

EFFECT OF INTERGEL® SOLUTION ON MORTALITY AND ABSCESS FORMATION AFTER INTRAPERIOTONEAL INFECTION IN RATS. STUDY REPORT. MAY 30, 2000.

STUDY TITLE:

EFFECT OF INTERGEL SOLUTION ON MORTALITY AND ABSCESS FORMATION AFTER INTRAPERITONEAL INFECTION IN RATS

STUDY NUMBER:

ETH4

TEST ARTICLE:

INTERGEL Solution
(0.5% Ferric Hyaluronate Gel)

IDENTIFICATION NO.:

H206APG0031Z

DATE:

May 30, 2000

SPONSOR:

LIFECORE BIOMEDICAL

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SUMMARY

The test article, INTERGEL Solution (a 0.5% ferric hyaluronate gel), was placed in the rat peritoneal cavity concurrent with the implantation of gelatin capsules containing rat cecal contents and barium sulfate slurry. This bacterial inoculum was expected to cause 50% mortality in the control animals based upon a pilot study. The number of rats that died as a response to the infection, and abscess formation on day 11 was evaluated and compared to control animals which received a similar volume of lactated Ringer's solution (LRS).

No difference was observed between the formation of abscesses and mortality when Intergel was placed in the abdominal cavity concurrent with a bacterial infection. Placement of 5 mL/kg Intergel in the peritoneal cavity concurrently with a bacterial inoculum did not affect the course of host resistance to the infection.

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INTRODUCTION

A host resistance model was used to determine whether implantation of INTERGEL Solution, a bioresorbable gel of iron-crosslinked hyaluronic acid, at the time of bacterial inoculation affected the mortality and abscess formation because of the infection. The purpose of this test was to determine if there was an increased risk associated with the use of this product in potentiating infection.

MATERIALS

Test Article:	INTERGEL Solution
Identification Number:	H206APG0031Z
Stability Testing:	Two Years
Expiration Date:	12/01
Storage Conditions:	Room temperature
Test Article Preparation:	None

METHODS

Animals:

One hundred twenty female Sprague Dawley rats, 175 to 225 gms, were used for the infection potentiation portion of this study (Onderdonk et al, 1974). The rats were acclimatized at least 2 days prior to surgery. The rats were housed in the USC Vivarium (an AALAC certified/accredited facility) on a 12:12 hour light/dark cycle. Food and water were available ad libitum except in the immediate postoperative interval.

Preparation of Gelatin Capsules:

The cecal contents and feces from rats fed hamburger for 2 weeks were collected and mixed 1:1 with nonsterile peptone yeast glucose broth containing no preservatives (Scott Laboratories) and 10% barium sulfate. The amount of this fecal preparation that caused mortality in 40 to 60% of the rats (LD_{50}) was determined in a pilot study (Table 1). In the pilot study, there were groups of rats that received inocula that resulted in 40% mortality and 60% mortality. The LD_{50} inocula was determined from this data, and the appropriate amount of material (125 µl) was aseptically added to a gelatin capsule (Number 1, Eli Lilly Company). The capsule containing the fecal preparation was then placed in a second larger capsule (Number 00, Eli Lilly Company). This was referred to as a double-walled gelatin capsule. The capsules were prepared up to 1 week prior to implantation and stored under frozen conditions under quarantine until the day of surgery.

Table 1. Mortality Data from Pilot Study

Volume of Feces	# Died/Total	% Death
25	0/5	0
50	0/5	0
100	2/5	40
150	3/5	60
200	4/5	80

Implantation of Gelatin Capsule:

The rats underwent a standardized procedure for laparotomy (intramuscular anesthesia with ketamine/rompum, shaving with animal clippers, betadine scrub, and alcohol scrub). A 2 cm incision was then made on the midline. A double-walled gelatin capsule was placed on the right side of the abdomen through the incision. In the control animals, lactated Ringer's solution (5 ml/kg), was instilled on the left side of the abdomen between the visceral and parietal peritoneum. In the animal treated with INTERGEL Solution (5 ml/kg), the material was placed on the left side of the abdomen between the visceral and parietal peritoneum.

The groups were as follows:

Group	Number of Rats	Bacterial Inoculum
Fluid Control (LRS)	60	LD_{50}
INTERGEL	60	LD_{50}

The abdominal wall and skin was then sutured closed using two layers of 4-0 Ethilon suture. Following surgery, the rats received analgesic for 3 days and were observed twice daily for signs of morbidity/mortality.

Necropsy:

The rats that died during the 11 day postoperative observation period were necropsied to confirm the presence of an acute bacterial infection. The rats that survived the initial acute infection were terminated on day 11 after surgery. Each rat was examined for the ability to palpate any abdominal abscesses through the skin, odor upon opening and splenomegaly. In addition, four areas of the peritoneum were examined for abscess formation. These areas included the liver, abdominal wall, bowel and omentum.

The abscesses were scored at each site as follows:

0	No abscess present at the site
0.5	One very small abscess present at the site
1	Several small abscesses present at the site
2	Medium abscess present at the site
3	Large or several medium abscesses present at the site
4	One very large or several small abscesses present at the site

The scoring was conducted in a blinded fashion by two separate observers and the scores recorded. If the scorers disagreed, the higher score was used.

STATISTICS: The sample size of 60 was calculated using the method described by Lachin for a comparison of two proportions. The assumptions were as follows: alpha level = 0.05 (two sided); beta level = 0.20 (80% power); control group mortality = 50%; treatment group mortality = 75%. These assumptions yielded a total sample size of 116 (58 subjects per group).

The data were analyzed by analysis of variance on the ranks (ranks of the scores of the individual and overall scores) or Chi square analysis (incidence of abscess free sites and mortality data). Comparisons which gave p values of less than 0.05 were considered statistically significant.

RESULTS

All rats at necropsy had abscesses that were palpable, splenomegaly and odor. There was no differences observed between groups.

No difference was observed between the formation of abscesses (Tables 1 and 2) and mortality (Table 3) when Intergel was placed in the abdominal cavity concurrent with a bacterial infection. In the group of rats treated with LRS, 36.7% (22 of 60) of the rats died during the period between bacterial inoculation and necropsy. In the group of rats treated with Intergel Solution, 40% (24 of 60) of the rats died prior to necropsy. The mean overall abscess scores for rats treated with LRS and Intergel Solution were similar (LRS 6.7 $\pm$ 0.25; Intergel Solution 6.02 $\pm$ 0.32).

CONCLUSION

Placement of 5 mL/kg Intergel Solution in the peritoneal cavity concurrently with a bacterial inoculum did not affect the course of host resistance to the infection.

Table 1. Abscess Scores in Rats Treated with lactated Ringer's solution

Liver	Abdominal Wall	Bowel	Omentum	Overall
2	2	0	2	6
3	3	0	1	7
2	3	3	3	11
0	2	1	1	4
0	4	3	0	7
3	0	1	3	7
1	2	2	3	8
0	3	2	1	6
1	2	1	2	6
0	2	4	2	8
1	2	4	3	9
0	2	0	1	3
0	3	2	3	8
1	3	2	1	7
0	2	2	1	5
1	0	2	3	6
3	0	2	3	8
0	2	3	2	7
0	2	1	2	5
0	2	2	1	5
0	3	2	0	5
2	0	2	4	7
0	3	2	1	6
0	2	4	1	7
1	2	1	2	6
2	3	0	3	8
2	4	0	1	7
1	3	2	1	7
0	4	1	1	6
2	1	1	1	5
2	1	0	4	7
0	2	2	1	5
1	0	2	3	6
0	3	2	1	6
0	2	2	3	7
3	2	0	3	8
3	3	1	3	10
0	3	3	1	7
50.8±4.5*	49.4±4.1	53.3±4.1	46.7±4.1	57.6±3.6

*These values are the mean and standard error of the mean of the ranks of the abscess scores at each site and of the overall score.

Table 2. Abscess Scores in Rats Treated with Intergel Solution

Liver	Abdominal Wall	Bowel	Omentum	Overall
1	2	0	1	4
1	2	1	1	5
2	4	3	2	11
0	2	3	2	7
0	0	2	2	4
0	1	0	1	2
0	4	0	0	4
1	2	3	3	9
2	2	0	1	5
1	3	2	2	8
1	1	0	1	3
1	4	0	2	7
0	4	1	2	7
1	4	2	2	9
2	3	2	3	10
3	1	0	2	6
1	1	0	4	6
1	3	0	3	7
0	1	1	3	5
1	2	0	3	6
0	2	2	1	5
0	3	1	1	5
1	1	0	2	4
0	3	1	2	6
1	2	1	2	6
0	3	2	2	7
0	3	2	3	8
2	0	2	3	7
0	1	2	3	6
0	3	0	1	4
0	3	1	1	5
2	2	1	1	6
0	4	2	2	8
0	1	3	2	6
0	0	4	1	5
0	2	1	1	4
44.0±3.9*	52.7±4.3	42.7±4.5	47.1±3.9	46.8±4.5

*These values are the mean and standard error of the mean of the ranks of the abscess scores at each site and of the overall score.

Table 3. Effect of Intraperitoneal Fluids on Survival after Peritoneal Infection

Group	# Died/Total	Percentage
LRS	22/60	36.7
Intergel	24/60	40.0

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Lachin JM. Introduction to sample size determination and power analysis for clinical trials. Controlled Clinical Trials. 2:93-112, 1981.

Onderdonk, AB, Weinstein, WM, Sullivan, NM, Bartlett, JG, Gorbach, SL. 1974. Experimental intra-abdominal abscesses in rats: Quantitative bacteriology of infected animals. Infect Immun 10:1256-1259.